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THE ULTRAMICROSCOPIC VIRUSES

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Aristotle wrote that nature makes so gradual a transition from the inanimate to the animate kingdom that the boundary lines which separate them are indistinct and doubtful. This thought is very pertinent when one considers the ultramicroscopic viruses. There appear to be *three possibilities as to the nature of the viruses:*

1. that the viruses are infinitely small organisms possessed of a nature similar to that of living entities of sorts already known.
2. that the viruses are inanimate transmissable incitants of disease.
3. that the viruses represent a form of life as yet unfamiliar to us.

In favor of the first possibility are the following facts:

A. The fundamental attributes of life, it is generally accepted, are metabolic assimilation of heterogeneous substances which are built up into the substances of the organism, reproduction, and adaptation. Viruses appear to have these properties. They have antigenic and therefore chemical specificity, increase in numbers, and change in response to differences in environment. It is to be noted, however, that these properties are exhibited only when the virus is associated with

living cells; it is therefore impossible to state definitely whether the virus is acting independently, or whether the cell under the stimulus of an inanimate substance elaborates more of the same substance from its own materials.

B. The frequent discovery of streptococci in the lesions of or cultures from poliomyelitis and encephalitis by Rosenow, and the experiments of Kendall on reduction of gross bacteria to ultramicroscopic dimensions and to the filtrable state and development of bacterial forms by transfers from the filtrates, have led to the thesis of Rosenow and Kendall that the ultramicroscopic viruses are the invisible, filtrable, cyclo-stages of ordinary bacteria.

These experiments do not provide convincing evidence that the viruses are the same as the ultramicroscopic filtrable forms of ordinary bacteria. This conception appears to be highly improbable.

C. The presence of inclusion bodies in invaded cells has led some to believe that the viruses are protozoal organisms which are regarded as one stage in the life cycle of which the filtrable virus represents another stage.

The viruses' high susceptibility, in common with protozoa, to saponin and bile salts has also led to belief in this theory.

In favor of the second possibility are the following facts:

A. The minute size of the virus may preclude its being a living thing.

B. Its apparent power of multiplication does not necessarily mean that it is alive, for it may be of the nature of an enzyme which in some way is increased in amount during the active process to which it gives rise. The virus protein molecule may be able to cause the smaller molecules already present in the cell or produced as a result of the presence of the virus, to combine to form a new virus particle.

C. The crystalization of the virus of tobacco mosaic disease by Stanley of the Rockefeller Institute has cast doubt on the possibility of the virus's being a living thing.

In favor of the third possibility is the fact that since so much doubt does exist as to the nature of the virus, it may be likely that our confusion is due to the fact that we are as yet unfamiliar with the form of life which the virus may represent.

Zinsser and Bayne Jones have summed up the possibilities in stating that circumstantial evidence leads to belief that the viruses are living, but the proof is not absolute.

Our definition of the virus must necessarily be a descriptive one. Topley and Wilson have defined the virus in the following manner: "Probably organized bodies which are usually invisible by ordinary microscopic methods of examination, which have a diameter of less than .2u, which can often be filtered through candles and membranes impermeable to ordinary bacteria, which have not yet been cultivated in cell free media, but which multiply freely in the presence of susceptible cells in vitro or in vivo, which generally have a high resistance to glycerol, which frequently invade one particular species of host and tend to affect one particular tissue, which give rise to characteristic inclusion bodies in the tissues, and which cause a latent or overt infection followed as a rule by a lasting immunity."

The first evidence that such an agent existed was discovered in 1892 through experiments of Iwanowski and Beijerinck on transmission of mosaic disease of tobacco with the cell-free filtrate of the juice of diseased plants. In 1897-1898 Löffler and Frosch found that the cause of foot and mouth disease in animals was a virus. The virus diseases have the characteristics of infectious diseases, yet no visible microscopical organism has been successfully demonstrated. Cell-free extracts used in transmission of the disease are most commonly obtained by filtration, therefore the specific infecting agents in the filtrate have been referred to as the filtrable viruses. Not all viruses have yet been shown to be filtrable. There is no exact quantitative standardization of bacterial filters and therefore the term "filtrable" is a loose one. Other names used for the virus are ultramicroscopic, protista, cheamydozoa, and strongyloplasma.

Methods of Examination for the Presence of a Virus

1. Filtration.

A filter is not a mere mechanical seive since there are other

factors such as the following, other than the size of the pore, which determine whether a given body will pass through:

a. Electrical charge on the filter.

b. Electrical charge on the virus.

Under ordinary biological conditions, in tissue fluids, virus particles carry a negative charge and can be concentrated at the anode by cataphoresis. At least the *virus bearing particle* has a negative charge.

c. Amount of protein or other substances in the virus emulsion and its electrical charge.

In protein solutions more acid than the isoelectric point of the protein, the dissociated protein is chiefly in the form of multivalent cations capable of combining with silicate anions in the filter and forms an insoluble combination which is retained. When the solution is more alkaline than the isoelectric point, the dissociated protein is chiefly in the form of multivalent anions capable of entering into combination with alkaline earth cations in the filter with the formation of soluble salts that pass through. This is probably why viruses appear to pass through filters more readily when in weakly alkaline solution than when in acid solution.

d. Adsorption of the virus by aggregates of protein or cell detritus.

e. Temperature at which filtration is carried out.

f. Amount of positive or negative pressure employed.

g. Duration of filtration.

The mere passage of an organism through a filter does not justify its inclusion in the group of filtrable viruses. Likewise, failure of passage does not exclude it from the group.

2. Ultrafiltration.

Thin collodion membranes prepared with a given size pore, therefore approaching more nearly the mechanical sieve, can be used to sort out particles according to size. Secondary factors are not so numerous here, but pH and electrical charge must still be considered.

The size of the viruses determined by this method has been shown to be very variable—from 5.5 mu for the virus of tobacco mosaic to 230 mu for vaccinia virus. This enormous range of difference may indicate that investigators have been measuring particles on which the virus was absorbed rather than the virus itself. Virus particles are within the order of protein molecules.

3. Microscopical examination with direct illumination by transmitted light.

   This method can be used provided the particles are not smaller than .25u in diameter, and provided they absorb sufficient light to render them visible. Absorption of light can be increased by impregnation with dye, but although satisfactory with larger particles, with those less than .2u in diameter the resulting image is almost entirely due to diffraction and therefore has optical properties independent of any color imparted to the object itself.

4. Dark field illumination.

   Lack of visibility of very small particles renders their examination by transmitted light useless. For this reason, dark field illumination is used to render the particles self luminous.

5. Photography by transmitted ultra-violet rays.

   This method may be used in order to obtain resolution of the particles which are merely rendered visible by dark field illumination.

6. Centrifugalization.

   It is very difficult to secure a machine sufficiently powerful to throw down very fine suspended particles since centrifugal force varies with the square of rotation and directly with the length of the arm; therefore it has not proved possible to throw down completely the filtrable viruses contained in cell-free suspensions. Sometimes by use of kaolin or animal charcoal added to the suspension it has been possible to concentrate a greater part of the virus in the lower layer, but this is not always so.

7. Methods of enzyme chemistry.

   Purification of the virus by selective adsorption on aluminum

hydroxide or other adsorbants followed by elution with phosphate solutions.

8. Animal inoculation.

A suitable animal must be selected. The filtrate is used for inoculation. The presence of the virus is determined by having the following conditions fulfilled:

1. Filtrate must produce the characteristic symptoms.
2. Filtrate must produce the characteristic lesions.
3. Filtrate must be free of bacteria.
4. Filtrate of the experimental lesion must again produce the disease.

9. Tissue culture.

The bacteria free filtrate is cultured in living tissue. The fertilized egg is commonly used for this purpose. The egg is incubated for 9-14 days for development of the embyro. The shell is sterilized, a window cut, and the filtrate placed on the choreo-allantoic membrane, the window is then sealed and incubation is continued.

Habitat of the Viruses

All the viruses known at present are associated with living cells. Their existence is essentially parasitic. It is not known whether saprophytic forms exist.

Many of the viruses in the animal body show a particular affinity for special tissues. However, even when lesions are confined to one tissue, the virus can also be demonstrated in other parts of the body. There is some evidence that tissue localization is more apparent than real, depending on the mode of infection. Many viruses exhibit species specificity giving rise to lesions in only one particular species.

A virus can be present in a healthy carrier free from all clinical symptoms; or it may remain latent in tissues after causing an initial infection. It is possible that an attack of intercurrent disease or some artificial procedure such as vaccination may activate such a latent virus and thus cause it to give rise to clinical disease.

It is not certainly known whether the viruses occupy an intra or

extracellular position in the body, but indirect evidence so far suggests their multiplication and growth occur actually within the cells. There is evidence that *viruses prefer young newly-formed cells for multiplication.*

Inclusion Bodies

Lesions in virus diseases often show on histological examination inclusion bodies in the cytoplasm or nucleus or in both. The appearance of these bodies varies in the different diseases. The bodies may be round, oval, pyriform, or irregular; they may be hyaline or granular; they may be homogeneous or contain elementary bodies; they may stain basic or acidic; they may contain lipoid substances.

There appears to be some relationship between the presence of inclusion bodies and the infectivity of the tissue. Inclusion bodies can be produced experimentally only by inoculation with the living virus. There is no doubt as to their significance. Their presence in tissue is a certain sign of infection and is made use of in routine diagnosis of certain of the virus diseases.

The exact nature of the inclusion body is not known. The following theories have been advanced:

1. Protozoal—varying stages of an elaborate life cycle.
2. Cellular degeneration products due to nucleolar extrusion, vacuolation of the cytoplasm, etc., consequent on the attack of the virus.
3. Microorganisms, elementary bodies, embedded in material deposited around them as a reaction of the cell protoplasm.
4. Aggregates of the virus itself.

In most cases the inclusion appears to be composed of both virus and cellular material.

Elementary Bodies

These minute bodies have been found in the exudates formed in certain virus diseases. Barrel found them in fowl pox in 1903; Paschen found them in vaccinial lesions in 1906. Since then they have been demonstrated in lesions of vaccinia, varicella, herpes zoster, fowl-pox, and psittocosis.

The bodies are agglutinated by serum of animals recovered from the homologous disease. Pathogenicity tests indicate that they are the etiological agents of the various diseases.

When stained (using prolonged Giemsa staining) they come within the limits of microscopical resolution, probably through the deposition of the stain on the virus.

Cultivation of Viruses

Cultivation of the viruses has never yet proved possible in the absence of living cells. (An exception to this is the virus of pleuropneumonia which has been cultivated in the absence of living cells). All experiments must therefore be made on a mixture of virus and cellular tissue. "Until the virus can be cultivated on artificial media and separated from all cellular material, progress in the study of their properties must necessarily be hindered by the presence of many uncontrollable and undetermined factors."

Resistance of Viruses

The resistance of the viruses seems to vary considerably. On the whole, they have the same degree of resistance as the vegetative bacteria, generally destroyed by moist heat at 55-60°C for half an hour and succumbing to fairly low concentrations of chemical agents. Their apparent higher resistance to chemical agents may be due to the presence of protein material in the medium.

Most viruses exhibit fairly high resistance to glycerol, and therefore glycerol is used for preserving infectious tissue. Glycerol probably inhibits autolysis of the tissue.

Metabolism of the Viruses

Very little is known. One great hindrance to the study of metabolism is the impossibility of cultivation in the absence of living cells. "Normal cells seldom break down carbohydrates with the formation of lactic acid even under aerobic conditions, or when they do, the amount of lactic acid formed is very small. Tissues infected with certain of the viruses which give rise to a considerable amount of hyperplasia do exhibit this aerobic glycolysis." We do not know if the viruses have metabolism of their own.

Pathogenicity of the Viruses

All the known filtrable viruses are pathogenic. The virulence is subject to great variation, for in adaptation to a new host the properties of the virus may be considerably altered. It is important in adapting viruses to new hosts to exclude the possibility of irregularities arising from the presence of spontaneous disease in the experimental animal.

The incrimination of a virus in any disease process is frequently very difficult. Reproduction of the condition in an experimental animal and subsequent passage of the infection are essential. *Failure to obtain passage of the infection indicates the action of a toxin rather than of a virus.*

Infection in most of the virus diseases appears to occur by direct contagion. The portals of entry include the skin, mucous membranes, and placenta. In certain diseases the virus may be inoculated directly into the blood stream by the bite of blood sucking insect vectors. A virus pathogenic by one portal of entry may be relatively avirulent when injected by another portal.

When a virus becomes active within a cell, it produces one of two types of lesions:

1. The cells may be stimulated to divide so that the tissues become hyperplastic.
2. The cell metabolism may be so severely damaged that rapid necrosis is inevitable.

The finer pathological processes in virus infections tend to center around a given type of body cell. This fact has led to one method of classification of the viruses.

Immunity in Virus Diseases

A single attack of the disease generally produces a solid and lasting immunity. The factors involved are likely the same ones involved in bacterial diseases.

Some investigators, such as Levaditi, believe that the immunity is largely a local tissue one. This local immunity may be independent of or actually dependent on humoral antibodies.

Humoral antibodies have been found with viricidal or neutralizing affect. In general, precipitins have been demonstrated more frequently than complement-fixing antibodies. Passive immunization is possible by the inoculation of antibodies.

Active immunization is now possible for variola, rabies, yellow fever, rinderpest, distemper, louping ill, feline enteritis, and fowlpox.

Only two virus infections, lymphogranuloma venereium and trachoma, give evidence that specific drugs are of value in treatment.

Once the virus has entered the body cells, it cannot be destroyed by the use of specific immune serum. Prophylactic immunization is therefore the best possible protection against virus disease.

It is very likely that many of our greatest problems in medicine reside in the realm of the ultramicroscopic virus. The answer to the etiology of cancer may lie there. The research worker in virology carries a heavy responsibility. May he have ability and vision.

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AN HYPOBROMITE METHOD, IMPROVED BY MEANS OF A NEW UREOMETER FOR THE DETERMINATION OF THE UREA-QUANTITY IN BLOOD AND URINE

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The hypobromite method for the determination of the urea-quantity in blood and urine, is nowadays largely discarded by the clinical laboratories in America. The same phenomenon has been seen in Europe. During many years the old hypobromite methods were justly rejected and ousted from the European laboratories. Then in America used methods came into vogue. New methods can be compared with film stars. It is for both easier to get in, than to get out through the large door.

The now most used methods for the determination of urea-nitrogen in blood and urine, are based on the hydrolysis of urea to ammonium carbonate by the urease ferment. Osgood recommends the Van Slyke and Cullen modification of Folin's ammonia-method, combined with Marchall's urease-method. To determine the nitrogen-content of ammonium carbonate, the ammonia is either distilled off or aereted into an acid medium, after which the ammonia content is determined. The latter is made either by Nesslerization or by titration. Karr's direct Nesslerization is a simplification, used in many hospitals.

Roger V. Dodson writes justly, that either of these methods are *time consuming and cumbersome,* especially in routine-work, where many determinations are made daily. (The Amer. J. of Technology, March, 1940, page 58.) To the same conclusions came the European technicians and physicians.

An important simplification is described by Roger V. Dodson in the above mentioned Journal.

Nevertheless it is necessary to use the urease-ferment and a

standard or time-consuming control estimations of ammonia in the urease and reagents.

The urease-ferment is a factor, which never can guarantee for 100%, as we never know, if the ferment is still sufficiently active. If the activity of the urease-ferment decreases, nobody knows, if not in one part of the used urease the weakening is more proceeded, than in another part. The consequence of this is, that the comparison with a urea-standard-solution in such cases cannot give any security.

As to the standard-solutions, all adepts know, that each standard requires "time consuming and cumbersome" control.

All these inconveniences and unreliabilities can be avoided by the use of an hypobromite method, improved by means of a new ureometer.

It has with certainty been proved a.o. by comparing with standard-solutions of urea and by comparing with the xanthydrol-method, (by which the urea is exclusively determined), that the hypobromite method, *if applied with reliable instruments*, gives *sufficiently correct results for the clinic*. (Chabanier, Becher, Schmid, Philiwert and myself). Accurate comparisons demonstrated, that this improved hypobromite method, was more reliable for clinical purposes, as not necessitating the use of less reliable factors (ferments and standards). Moreover it is simple, after a little exercise, and more apt for the general practitioner, who looks also at the question from the economic angle.

The use of more ureometers at the same time, in larger hospitals, makes it possible to achieve the urea-determinations much more rapid, than it can be made by the urease-methods.

It would carry too far, to discuss other less used methods, that have superseded the old hypobromite methods, which were indeed very bad. Therefore I advise anyone, who is not fully contented with the method, now used by himself, to try the new ureometer and not to condemn it, on ground of bad experiences with obsolete hypobromite methods. Only in that way, it is possible, to pass a judgment of value.

Hyperazotaemia of renal or extrarenal origin, is a symptom of such importance for the diagnosis and therapeutics, that the method, with which the blood-urea quantity is determined, should draw close attention.

I have tried to construct a new simple ureometer for the hypobromite method, the use of which removes the great failures, inherent to the older models of ureometers. In order to put this in a clear light, I shall first point out these failures, adherent to the older models. The model used most, is the Ambard-Hallion ureometer, a modification of Yvon's classic model. As to this apparatus, with which I have worked for years, close attention must be paid to the airtight fitting of the rubber bags, when the tube is filled with fluid and gas. Some of these rubber bags fit closely round the glass tube, but others may soon cause trouble, air entering the tube through the ill fitting rubber bags. Less dangerous are great leakages, for they are seen at once. On the other hand, small leakages may have existed for a long time, before being detected. Many results proved to have been unreliable, so that the diagnoses were inaccurate.

In the last ten years, I used to repeat, if possible, every examination on urea in blood or urine, after some days, in order to control the reliability of the methods. By a wrong agreement of the results in those examinations, the failure was often caused by the abovementioned defect. Hence it would be of great value, when, in applying the hypobromite method, the rubber bags could be dispensed with.

The Ambard-Hallion tubes are unfit for the examination of small quantities of blood. For this purpose, Ambard has put on the market a so-called micro-ureometer. With this apparatus 1 or 1½ c.c. of blood can be examined, which is of importance, e.g., for series-examinations or for examining children. Ambard's micro-ureometer consists of a thin graduated tube, of which the lower end is widened. In the reservoir, with cock and rubber bag, attached to the glass tube, the gas is envolved, which is then collected under water in the thin tube. Experimenting with this apparatus, I often failed to make the gas rise in the narrow tube, even if I used the brass wire, added for this purpose. A worse fault is that, owing to a leaking rubber bag, the whole experiment may prove to have been useless. Especially if one works with small quantities of blood, a not perceptible leakage may be the cause of great mistakes in the results.

Years ago, I was present at a congress for internal medical science at Wiesbaden, where Citron's ureometer was recommended. I made experiments with this apparatus, too. The objection I have

against this apparatus, is, that the smallest leakage in the rubber-stopper-closure, immediately results in great mistakes. The substitution of rubber-stoppers for rubber bags, did not appear to be an improvement.

With an ingeniously constructed azotoscope, Terwen tried to examine small quantities of blood, with the aid of the hypobromite-method. He used rubber stoppers, a characteristic of Citron's apparatus, but he struck on the same rock. Experimenting with this azotoscope, I was often troubled with leaking stoppers.

Kowarsky seemed to know this impediment, for he constructed an ureometer without either rubber bags, or rubber stoppers. I also made experiments with this apparatus, but, in doing so, I met with other difficulties. Firstly the removal of an air bubble, often sticking under the three-way-cock, requires a juggler's adroitness. Another objection I have against this apparatus, is, that it is impossible to roll the apparatus between the palms of the hands, so that the small gas-bubbles, adhering to the glass-sides, can hardly be loosened. Lastly, that the intricacy of the apparatus (threeway-cock, double tube) makes it expensive, whereas the breakable construction may cause disastrous injuries. Quite another difficulty in using this apparatus, was, that, on account of the relatively too low specific gravity of the salt solution, which is used with it, the enclosure of the gas evolved, is not reliable. Discussing the new ureometer, I shall give further details about this difficulty.

Another trial, to avoid the rubber fittings, consisted in constructing an ureometer, in which an attempt was made, to obtain the enclosure of the gas evolved, by means of a marble. Experiments, however, showed me, that this enclosure is often unreliable.

With this I have, in broad outlines, explained the difficulties, inherent to the most used ureometers.

The new ureometer, (fig. 1) gives reliable results, partly founded on the following advantages:

1. Absence of either rubber bags or rubber stoppers, eliminating leakage-risk.

2. Use of the atmospheric pressure, and of a salt solution, the specific gravity of which, is much higher, than that of the hypobromite-solution, and of the 20% trichloracetic acid, in order to enclose the evolved gas in a purely physical way.

3. Such construction as to make it easy, to loosen the gas bubbles, sticking to the glass side, by rolling the apparatus between the palms of the hands.

4. Simple construction, so that the price is low.

5. Strong construction, so that there is little danger of breaking it.

As mentioned above Kowarsky used a salt solution for the enclosure of the gas evolved, but the specific gravity of this solution was still too low, to ensure a reliable enclosure. The solution of hypobromite of soda, prepared with 33% sodium hydroxide, did not form with Kowarsky's solution a sufficiently sharp borderline, but was often seen to mix soon. This agrees with the specific gravities of these solutions, which differ too little. No doubt, it would be an error, to lower the concentration of the hypobromite solution. The effect of this is, as I was able to state by means of experiments, that a smaller part of the urea is decomposed, so that, the results cannot be compared anymore with those of the hypobromite method, in which 33% sodium-hydroxide solution is used. So it was necessary to prepare a salt solution, the specific gravity of which was *much higher*, than that of the hypobromite solution, prepared from 33% sodium-hydroxide. In this we have succeeded, by the aid of a solution of NaBr, after numerous other salt-solutions proved to be unfit for that purpose. It is prepared in this way: one weighs off 115 gm. of NaBr, and add 130 cc. of water. Dissolve this by gentle heating. The seemingly higher cost, by using a solution of NaBr, compared to other salt-solutions is avoided, because it is very easy, to draw off the NaBr-solution after the examination. In this way at most 4 to 5 cc. of the solution are lost in each examination, which costs next to 1 cent. The specific gravity of the hypobromite-solution is at 16 x dilution 1018, and that of the NaBr solution, used by us, is at 16 x dilution 1029, whereas the salt solution used by Kowarski (35% NACL + 15% K2SO4) at 16 x dilution, has a specific gravity of 1019 only. With the above-mentioned NaBr solution, one obtains the perfect enclosure of the gas. A NaBr-solution should be preferred to a KBr-solution, as the NaBr salt dissolves better in water; viz., 1 part in 1/15 part of water, whereas of KBr, 1 part dissolves in 1/6 part of water. Therefore, solutions of higher specific

gravity can be better prepared from NaBr than from KBr. Stronger concentrations than 115 grm. of NaBr + 130 cc. of water, crystallized out in the cold, after twenty-four hours. They are moreover entirely superfluous, because the specific gravity has been raised more, than was necessary, in this concentration.

It is advisable to begin the investigation with the preparation of the solution of hypobromite of soda (NaBrO), because it takes time to let this cool down, before it can be used. Knowing, that mistakes are being made, in preparing the NaBrO solution, I shall give a few directions for a reliable mode of preparation.

One should have in readiness a sodium hydroxide-solution of about 33% solution, e.g., by weighing off 50 grams of sodium hydroxide (NaOH), pure, in sticks, (hydras natricus alcoholicus depuratus in bacillis) and by adding to this 100 cc. of water. I rejected the use of a KOH-solution, because this may give rise to turbidity, when brought into contact with the NaBr solution. Bromine can be kept in double-stoppered bottles. The NaBrO-solution, suitable for the purpose aimed at, is prepared, by adding one part of bromine to ten parts of 33% NaOH-solution. By using an acid-chest or the so-called thermopipette, we escape the trouble of the bromine-vapours.

If more than one part bromine is added to ten parts of 33% NaOH solution, a part of it remains undissolved, and by adding too little bromine, the urea is not decomposed sufficiently. This NaBrO solution, provided it be kept in a dark cool place, remains fit for use for, at most, one day. Should older NaBrO solution be used, then part of it has, by oxidation, been converted into $NaBrO_3$ (according to the equation $NaBrO + O_2 = NaBrO_3$), which does not serve our purpose. The NaBrO solution having been prepared, one should wait, until the mixing has cooled down to room-temperature, and until the initial evolution of gas-bubbles has subsided. It is therefore advisable, to begin with this part of the examination, as the cooling down will certainly be sufficient, when one got ready with the other preparation.

Although with the new ureometer, readings can be made, using about 4 cc. of blood, yet I strongly advise, for the sake of greater reliability of the results, to do, if possible, a blood-letting of at least 6 and preferably about 14 or 15 cc. of blood. As a rule one should

take 15 cc. of blood.[1] The more so, as nobody will object to a somewhat greater bloodtaking. In the great majority of cases, also in children, it is possible, to take 6 or more cc. of blood. If, however, this is not possible, we must have recourse to the much more time consuming and cumbersome micro-methods.

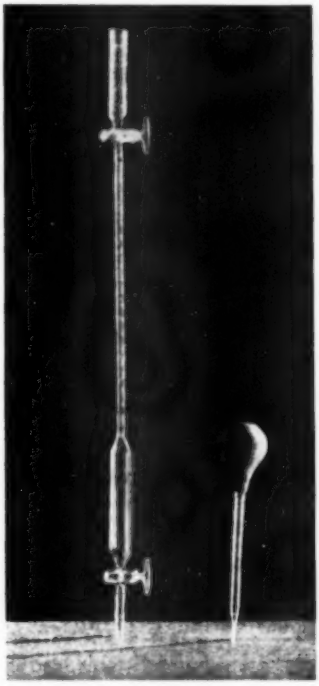

FIGURE 1

[1] Osgood recommends as quantity of blood, needed for the urease method 5 + 1 = 6 cc. and "still better" 5 cc. more = 11 cc. blood. Laboratory Diagnosis, Third Edition, p. 422.

To the blood, kept fluid by mixing it with some crystals of sodium oxalate, one adds equal parts of a 20% aqueous solution of trichloracetic acid. Shake and filter this. Using about 15 cc. blood, we shall have more than 11 cc. blood-trichlor-filtrate, using 6 cc. blood about 4½ cc. filtrate. During this filtration, a siphon-tubing or a syringe (one of both supplied with the apparatus) is filled with the NaBr solution (prepared in the above-described way). The ureometer, of which both cocks are opened, and the siphon-tubing, are fastened between the clamps of a standard. One fills the ureometer from below by the aid of the filled siphontubing or the filled syringe. The free end of the rubber-tubing of the siphon-tubing or syringe is adjusted over the lower end of the ureometer. The ureometer is filled up with the NaBr solution, until the tube is filled up to a little above the upper cock. Then this upper cock is closed, and the rubber tube is detached from the lower end of the glass tube. By means of the atmospheric pressure the fluid remains in the tube. With a pipette with rubber bag (supplied with the apparatus) the NaBr solution, left above the upper cock, is then entirely sucked away.

Experimenting with a new ureometer, one should first verify with a reliable pipette, whether the graduations on the vessel above the upper cock are accurate. These proved to be correct, on the tube verified by me. Then the blood-trichlor-filtrate is poured into the vessel above the upper cock, just up to the mark 11, if we have more, than 11 cc. filtrate, what will be the case, if we have the disposal of 15 cc. of blood. Before opening the upper cock, a clean basin is placed under the lower opening of the ureometer, to catch the running NaBr solution, which can be used for another examination. By cautiously opening the upper cock, so much of the blood-trichlor-filtrate is allowed to pass under this cock, that *exactly* 1 cc. is left above it. Then the upper cock is closed. So 10 cc. of blood-trichlor-filtrate are found under the upper cock. With less, than 11 cc. filtrate, we pour the whole available filtrate in the vessel, read then the quantity, take care, that after opening the cock, we close this cock, as soon as 1 cc. of the filtrate is left above it and note the quantity then found under the cock. The superfluous filtrate above the cock, is drawn off with the above mentioned pipette and the vessel is rinsed with water, by the aid of this pipette, in order to remove

practically all urea above the cock. This rinsing with water is necessary, but one rinsing will be sufficient. I found, that the neutralization of the blood-trichlor-filtrate with NaOH, recommended in several laboratory books, may be left out, since I purposely increased the quantity of NaBrO-solution added, to neutralize the filtrate. One pours therefore about 11 cc. of NaBrO-solution into the vessel above the upper cock, open the latter cautiously, making sure that a basin has been placed under the tube of the ureometer, to catch the running NaBr-solution, which as said above, can be used for another examination again. So much of the NaBrO-solution is allowed to pass under the upper cock, that 1 cc. is left above the cock, then the upper cock is closed. Less than 10 cc. of NaBrO-solution would suffice, although a slightly increased quantity will do no harm, whereas a decreased quantity can produce this effect, that a part of the urea is not decomposed sufficiently. The used NaBr-solution, as mentioned above, has a much higher specific gravity than the blood-trichlor-filtrate and the NaBrO solution. The mixing of the latter and the blood-trichlor-filtrate is complete. The high specific gravity of the NaBr-solution makes that a sharp borderline arises and that the gas evolved is safely enclosed. During the evolution of gas, the lower cock is left open. Else the upper cock is put to an unnecessarily severe test, as regards its obturation.

The gas, evolved, displaced the NaBr-solution and not until the evolution of the gas has practically ceased—i.e., after five minutes—the lower cock is closed. The ureometer is detached from the clamps of the standard. One rolls the widened lower part between the palms of the hands, so that gas bubbles, sticking to the glass wall, are set free and added to the already collected gas. Then the ureometer is fastened again between the clamps, after which the lower cock is opened again. The gas is often seen to expand a little yet. After waiting again for about five minutes, one reads off the results. So after about ten minutes, the whole evolution of the gas has ended. Should one not close the lower cock, while rolling the tube between the palms of the hands, a part of the NaBr-solution may be lost, which causes erroneous results. It is not necessary to place the ureometer up to the liquid borderline into water, when reading off the volume of gas. This does not give differences of any importance, if only one takes care, that the lower cock is always open,

when reading off the volume of gas. The relatively quick reading, i.e., ten minutes after the beginning of the evolution of the gas, is only allowed, on the condition, that one should work with concentrations of urea, as they are found in the blood-trichlor-filtrate. In order to be able to work with the same tube of the ureometer, for the determination of urea in urine, one makes a dilution of the urine, so that the concentration of urea in urine, vary within the limits of the concentration of urea of the blood-trichlor-filtrate. Not all nitrogen of the urea is freed by the hypobromite solution. Traces of gases of other origin are also formed. By that the error is compensated so far, that *for practical application in the clinic*, one may make the calculation, as if all the measured gas consisted of nitrogen, which has arisen from urea. Working at an average room-temperature (about 60°-70° F.) and an average height of the barometer, one may, in using the new ureometer, practically admit that 1 cc. of nitrogen arises from 2.7 mg. of urea. *Many experiments made with verifying solutions of urea and use of the new ureometer, have confirmed this.* According to the calculation from the chemical formula, 1 cc. of nitrogen arises from 2.68 mg. of urea, at a temperature of 0° and 760 mm. height of the barometer. The volume of nitrogen, read off, at an arbitrary temperature and arbitrary height of the barometer, can, by the aid of the well-known formula for the reduction of gas, (deduced from the laws of *Boyle-Mariotte* and *Gay-Lussac*), be converted into a volume at 0° temperature and 760 mm. pressure. The loss of time, in calculating this formula, can be shortened by the use of tables. Temperature has a greater influence than height of the barometer. The temperature of laboratories, being kept between about 60°-70° F. by the laboratory experimenters themselves on account of the unpleasant feeling of either cold or heat, one is allowed to take, *as a standard for the daily practice*, the empyrical value, above-mentioned.

By experience I found that acidum uricum, creatine, etc., in consequence of their low concentrations, have *no practical influence* on the results. If one will make a verifying experiment with a solution of urea, at an average room temperature and an average pressure, a 1:1000 solution of urea may be used, which must be freshly made. Of this 3.7 is measured off, with a pipette and (after introducing the NaBr-solution), poured into the ureometer

above the upper cock. By opening the upper cock, so much is allowed to pass below the upper cock, that 1cc. is left above this cock, So 2.7 cc. of the 1:1000 urea-solution are found under the upper cock. For the rest one acts accurately as described before (rolling between the palms of the hands, etc.). Under the above-mentioned conditions 1 cc. of nitrogen evolves. Should this not be the case, one should look for the cause of the difference, by the aid of the data, described before. The reading must also take place about ten minutes, after the evolution of gas has begun. Ten to fifteen minutes after the evolution of gas has begun, the readings were still just the same. A reading after a shorter time, than ten minutes is not reliable. If, however, one should wait longer than fifteen minutes, oxygen may be liberated from the NaBrO, by which, quite independently of the urea-nitrogen, the volume of gas increases. Often too little attention was paid to this source of errors, by which examinations have become unreliable. In using the new ureometer, and in applying the afore described method, one should be tenacious, that the reading is made between 10-15 minutes after the evolution of gas has begun, as the quantity of nitrogen to be expected from a verifying solution of urea, is found after this space of time.

As regards the examination of urine, special attention should be paid to the fact, that in older urines, part of the urea has often been converted into ammonia. Therefore, fresh urines must always be used or urines, to which, in a fresh condition, some chloroform has been added, to prevent ammoniacal fermentation under the influence of bacteria.

The examination of the urine can be performed with the same tube of the ureometer, provided the urine has been diluted, as has been mentioned, to the extent that the concentration of urea lies approximately within the boundaries of the urea-concentrations of the trichlor-blood-filtrate. Previously one should ascertain, whether the urine contains more than traces of albumen. If it is free from albumen, the urine is diluted one hundred times. The simplest method is to place a ½ cc. of urine into a 50 cc. cylinder-glass. Make up this quantity to 50 cc. with water. Shake well. Of this quantity pour 11 cc. into the vessel of the ureometer above the upper cock as described for the blood-trichlor-filtrate.

If the urine contains more than traces of albumen, one should

proceed as follows: One measures out 3 cc. of urine; add to this 3 cc. of the 20% trichlor-acetic-acid-solution, filter away the albumen. Take 1 cc. of the filtrate. Pour this quantity into a 50-cc. cylinder glass and make up with water, to 50 cc. Thus the urine is also diluted one hundred times. Of this quantity place also 11 cc. into the vessel above the upper cock of the ureometer. Proceed as described before.

Calculation. Working at an average room-temperature and an average height of the barometer, we may, as mentioned above, *practically* admit that 1 cc. of nitrogen arises from 2.7 mg. of urea.

Should in exceptional cases room-temperature and height of the barometer differ greatly from the average, the simplest method will be to use the above mentioned fresh-made solution of urea 1:1000, as a standard for the calculation. If only the room temperature greatly differs from the average, it is still simpler to put off the examination till the temperature in the laboratory is tolerable.

We call C. the number of grams urea per litre of urine and Ur. the number of grams urea per litre of blood. If one reads off a cc. of gas, and if one has started from b cc. of urine or blood, C. or Ur. is equal to 2.7 x a/b.

Supposing that in the ureometer, below the upper cock, is 10 cc. of blood-trichlor-filtrate, i.e., 5 cc. of blood. Supposing that this 5 cc. of blood produces a cc. nitrogen-gas. This nitrogen-gas has arisen from 2.7 x a mg of urea as 1 cc. of nitrogen-gas arises from 2.7 mg. of urea. Then 1 cc. of blood will contain 2.7 x a/5 mg. of urea and 1 litre of blood will contain 2.7 x a/5 gram urea or Ur = 2.7 x a/5, which agrees with the formula 2.7 x a/b. Supposing that the below the upper cock is not 10 cc. blood-filtrate, but only p cc., i.e., p/2 cc. of blood. In that case Ur = 2.7 x a x p/2.

The following example may serve for the calculation of the urea-quantity in the urine. Supposing that in the ureometer, below the upper cock, is 10 cc. of the 100 x diluted urine, i.e., 0.1 cc. urine. Supposing that this 0.1 cc. urine produces a cc. of nitrogen-gas. This nitrogen-gas has arisen from 2.7 x a mg. of urea. Then 1 cc. urine will contain 27 x a mg. of urea, and one litre of urine will contain 27 x a gram of urea, or C = 27a, which agrees with the formula C = 2.7 x a/b.

For all calculations, with which a physician has to deal, I have designed a pocket-slide-rule. At my request this pocket-slide-rule will be obtainable, with instructions, at the Laboratory Appliances Co., E. Machlett & Son, 220 East 23rd Street, New York. This slide rule is especially suitable for physicians who make laboratory investigations and gives answers, correct till 0.5%, within one minute, for all more or less complicated laboratory-calculations, without the use of pencil and paper. On the back of this slide-rule the normal values for the most used examinations of blood, urine and gastric contents are mentioned.

For urea-investigation, one should have in readiness:

1. A 20% aqueous solution of trichlor-acetic-acid.

2. A sodium-hydroxide-solution of about 33%, prepared by adding 50 gram of hydras natricus alcoholicus depuratus in bacillis to 100 cc. of water.

3. Bromine.

4. A solution of brometum natricum, prepared by adding 115 gram NaBr to 130 cc. water, dissolved by gentle heating.

5. Oxalas natricus (sodium oxalate) in crystals, to keep the blood fluid.

AN IMPROVED BLOOD TYPING TOOL*

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The blood transfusion has been aptly described as that surgical operation which is more symbolic than any other of the brotherhood of man. Granting the validity of such a description, the selection of the Donor then imposes a responsibility which is of a moral and religious, as well as technological, nature, and the fact that this responsibility now almost universally is delegated to the technologist should leave us with a sense of pride in our profession and a determination to give more original thought and attention to this problem than the minimum required to enable us satisfactorily to carry out the technical details involved in the selective procedure in a mechanical way. It further implies that we should be permitted certain temporal and material facilities by those who have delegated this responsibility to us toward an advance upon the unknown factors involved.

The Brice Blood Typing plate represents a tool or material facility. As in the case of other such tools, necessity was the mother of its invention. In the institution in which my education in the technical details involved was begun, we carried through the selective procedures by the microscopic methods. Subsequently I accepted the principal technological responsibility of the Clinical Laboratory of another institution where I learned of the Cornell doctrine with reference to donor selections that "nothing must come between" the blood specimens under cross agglutination examination. We defibrinated the bloods mechanically, separated them into component parts by centrifuge, and cross agglutinated, Donor's against Patient's, macroscopically, on individual 3 x 1 inch glass slides. The interns of the institution gathered around and helped with the

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veinipunctures, the defibrinations, at which the donors themselves also assisted, and the cross agglutinations. One day the interns were all called away to other duties and I found myself with the patient's blood specimen and the specimens of a large number of friends and relatives, each anxious to act as the donor. The patient was hemorrhaging in the operating room. The surgeon sent a student nurse who appeared at the door of the laboratory every few minutes with the question "Mr. Brice, have you gotten a donor yet?" A piece of scrap ¾" wooden board about 6" wide and a foot long was lying on the floor under the sink. I picked it up and racked a number of slides on it so as to oscillate the cross agglutinations of a number of donors' specimens simultaneously and the Brice Blood Typing plate came into being.

On first taking up the macroscopic agglutination procedure my reflexes were condition to a ½ hr. period of observation of diluted blood specimens under the microscope. While it seemed obvious that undiluted specimens resulting in proportionately higher concentration of the unknown substances involved in these reactions should require, at any given temperature, shorter time periods for the consummation of the reactions, still some factual basis of information of the time element involved seemed desirable and I therefore timed by stop watch several hundred of these reactions for a full half hour period. The maximum time period observed in but two to three per cent of the reactions, from the initial mixing of the specimens to the first macroscopically definite appearance of positive agglutination was 6 minutes. The arbitrarily established period of 7 minutes originally specified has seemed scant insurance against the possibility of a weak or delayed reaction being overlooked, so for some years I have been oscillating all reactions for a full 8 minutes. At the present writing I see no reason for further lengthening this period. On rational as well as technological grounds the macroscopic procedure with undiluted specimens has always and still seems to me to be preferred over the microscopic methods. The dilution of the unknown physical or chemical agents designated as agglutinogens and agglutinins can hardly result otherwise than in the time and temperature factors being more critical than they are when these agents are brought into reaction with one another at the full concentration in which they are present in vivo. The continuous

oscillation of the mixtures for the full period of time prescribed, simulating the movement that would take place were the mixture effected within the patient's vein and bringing every erythrocyte into presumptive contact with every other erythrocyte in an environment consisting of the opposite blood serum, seems technologically a more valid anastamosis than more or less protracted observation under the microscope of a similar but more dilute mixture in a state of rest.

Being somewhat scientifically agnostic we may still question the validity of the "nothing must come between" doctrine, and wish to know if its promulgation were based on any observation of fact, and if so what. In pursuance of such a thought I have run parallel cross agglutinations by the macroscopic procedure outlined. The series consisted of 27 individual cross-matchings done in duplicate. The patient's specimens of which there were eleven were taken by veinipuncture and defibrinated, as were also those of the 27 donors. Additional specimens were taken from each of the donors by finger prick into citrated capillary tubes which were sealed with wax and the cells and plasma subsequently separated by centrifugation. The donor selections were made from the defibrinated specimens and the donors certified to the surgeons. Thereafter when time permitted the parallel cross agglutinations with the citrated specimens were also run. There was perfect agreement between the results with but a single exception. Eight donors' specimens were cross agglutinated for transfusion to a single patient and four of them found to be suitable. One of these was selected by the surgeon and taken for the transfusion. While the transfusion was in progress the citrated specimens were separated and the parallel series run. There was agreement with the results of the defibrinated series with the single exception of the donor who had been selected and was being usd in the transfusion. This specimen showed definite agglutination appearing during the 5th minute of oscillation in the mixture of the patient's cells by defibrination with the donor's serum by citration. The surgeons were at once notified but the transfusion had been completed. Two of the surgeons came to the Laboratory and witnessed this phenomenon. The cross agglutinations were repeated with identical results. The patient's reaction to the transfusion was reported to me as "marked, with chills and high fever." My notes made in the Lab. on this occasion are dated Dec. 27th, 1928.

Of course scientifically valid conclusions from such a single observation cannot be drawn; however, it has left an impression. The question as to whether the agglutination phenomenon observed when citrate "came between" was an instance of anomalous or false agglutination cannot be answered, but when as and if the isoagglutinogens and isoagglutinins may ever be isolated and chemically identified, I believe that they will be found to be closely associated with, if not an actual part of the chemical complexes involved in the clotting phenomenon.

The original model of the Brice Blood Typing Plate supplied to me in 1928 by the Arthur H. Thomas Co. has worn well, and I have long since ceased to keep the record of the number of cross matchings and donor selections at which it has assisted. About a year ago, however, the repeated puddling of the cells and serum mixtures with the glass rod not moving always within the allotted area resulted in the wearing off of the lines of bitulithic paint, designed to keep the drops from merging, at a number of points, and a search for a more durable material was undertaken. On Treasure Island in San Francisco Bay at the Golden Gate International Exposition the DuPont Ceramic Colors were found. The plate is now supplied with area division lines of this material which is fused into and becomes an integral part of the glass by a technical process of "firing" at high temperature, so that barring breakage the plate may be considered a lifetime instrument. The symbols "A" and "B" as well as "PC + DS" and "PS + DC", have been added to assist the technologist and obviate explanations and notes in cases where he may be required to submit the reactions to his supervisor for reading or approval.

In a busy general hospital where the best surgical practice calls for the selection of blood donors before many operations are undertaken a number of such selections may often be called for in a single working day. The requests for donors drift into the Laboratory and the patients' specimens are collected during the morning. The stock known type isoagglutinating sera are taken from the refrigerator and allowed to come to room temperature. The specimens and known sera may all be brought to the plate and the whole cross-word puzzle of patients' types worked out in a few minutes. The secretary calls the requisite number of donors of the requisite

types from the Laboratory Donor List and their specimens are taken and separated. The plate is again called into use and the individual cross agglutinations are run. The donors' sera go to serology for the Wasserman or Kahn exclusion tests and as far as the Laboratory is concerned, the surgery for the next day has been set up.

In offering the improved model of the plate thanks are due to the representatives of the DuPont Chemical Co., the Dillon Color Glaze Co. of San Francisco, and the Arthur H. Thomas Co. of Philadelphia whose co-operation has made it possible.

1. Approved Laboratory Technic. Kolmer and Boerner. 2nd Edition Appleton-Century Co., New York, 1938, page 611.

COMPLEMENT FIXATION AND FLOCCULATION TESTS WITH THE SAME ANTIGENS

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As such a variety of satisfactory blood tests are available for use in the diagnosis of syphilis, it would seem wise to select the ones which would give the most information with a minimum of time required.

The Kline, Hinton and Laughlen are relatively simple procedures and are quite dependable if at least two methods are used together.

There remains some place, however, for a complement fixation test as this additional information is of value.

In about 98 per cent of bloods, if the tests are made in the first twenty-four hours after the serum is secured, there is sufficient native antisheep hemolytic amboceptor, and native complement to perform a test in a relatively simple manner. The hemolysins present are both thermolabile and thermostabile.[1]

There are some advantages in using active serum as both the thermolabile and thermostabile factors are kept. Noguchi[2] showed that in some individuals the thermolabile factor constitutes the greater percentage of the syphilitic antibody.

Use 0.3 cc. of unheated serum adding .05 cc. of the Kline, Hinton or Laughlen antigen, incubate one-half hour, then add 0.2 cc. of 4 per cent suspension of sheep cells in normal saline solution and incubate a second half hour. For each blood a control is used to show where there is anticomplementary action of the serum or a lack of hemolysin. Where hemolysis is present in the control tube and absent in the tube containing the antigen, the reaction is positive. Gradwohl[3] titrates this hemolytic index before using a similar method with the Noguchi antigen, but this makes the test too complicated for rapid work.

This test is not safe or satisfactory to use as a single test, but

where it is used in connection with two or more flocculation methods it is of value and requires very little extra time, as it makes use of the same antigen and can be performed while the serum is being heated for the flocculation test. In this way they can be read together.

A very satisfactory combination is to use this method of complement fixation with 0.3 cc. of unheated serum and inactivate an additional 0.6 cc. of serum, using about 0.1 cc. for the Kline and Laughlen. Read all three tests and then add 0.5 cc. of the Hinton antigen suspension to the 0.5 cc. of the serum remaining and leave in the incubator or water bath over night.

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THE "WHIPPING" METHOD, ITS COMPARISON WITH OTHER METHODS IN MAKING EXAMINATIONS FOR MYCOBACTERIUM TUBERCULOSIS

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Introduction

Every laboratory worker who attains a high degree of efficiency, employs every available aid in making his examinations. Some methods are too time consuming, and others too costly to be used routinely. Just how much time or money should be spent in making a test, depends entirely upon the individual case and the importance of the laboratory report. In making a sputum examination for mycobacterium tuberculosis, sometimes nothing is too expensive or laborous, in order to establish a positive diagnosis.

Historical

Several years ago, a young physician* suggested the "whipping method" as an aid in testing sputa for acid-fast bacteria. He had used this method as an interne, and believed that he had obtained more positives with it than he had with the regular "picking" method; i.e., picking out a most likely portion or particle. The method consisted in wrapping a small amount of cotton on the end of a wooden applicator to make a small swab, then whipping the sputum rather vigorously for some time. The swab was then applied, rather lightly, to the surface of a microscope slide, rotating as it was smeared along the surface to make a thin smear. He had not, however, worked out the length of time to beat the sputum in order to gain the best results.

Purpose and Scope

Believing that the "whipping method" would be an aid in making sputum examinations, and a comparison of its efficiency with other methods would be of interest, this work was undertaken. It consists in two parts:

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1. Development of the "whipping method",
2. A comparison of its efficiency with other common procedures.

Part I, Development of the "Whipping Method"

A small amount of cotton was wrapped on the end of a wooden applicator to make a small swab. One hundred sputa, positive for acid-fast bacteria, (previously tested), were whipped vigorously for one, two, three, four, and five minutes. The swab was then applied, rather lightly, to the surface of a microscope slide, rotating it as it was smeared along the surface, to make a thin smear. The smear, thus made, was dried, fixed, stained by the Ziehl-Neelson method, and examined for tubercle bacilli under the microscope. The results obtained were as follows:

Whipping for 1 minute	86 positives
Whipping for 2 minutes	99 positives
Whipping for 3 minutes	100 positives
Whipping for 4 minutes	100 positives
Whipping for 5 minutes	100 positives

From the above results, it is evident that whipping for longer than three minutes does not increase the efficiency of the whipping process; also that it does no harm. In view of this fact, three minutes was chosen as the whipping time, when this procedure was employed.

Part II, Comparison of the "Whipping" Method with Other Methods

Procedure: In this part of the study, 2,571 sputa sent to this laboratory for routine examinations were used. Out of this number, 1000 were found to contain Mycobacterium tuberculosis, in one or more ways. In rating the efficiency of the various methods, a sputum found to have acid-fast bacteria in it, (in any manner), was placed in the "positive class." The following methods were used:

1. The "Whipping Method"; whipping the sputa for 3 minutes, as described above.
2. The "Picking Method"; i.e., picking out a most likely portion.
3. The "Concentration Method"; Petroff's sodium hydroxide, A.S.C.P.
4. The "Culture Method"; Corper's potato medium, Corper and Uyei, (1928), Corper and Uyei, (1929), Corper and Uyei, (1930).

A microscope slide was divided into one-half, with a wax pencil.

On one side a smear was made, using the "picking" method; and on the other side a smear was made, using the "whipping" method. After drying, they were fixed, stained by the Ziehl-Neelson method, and examined for tubercle bacilli. After three minutes of the whipping process, most of the sputa were fairly well mixed into a homogeneous mass. However, those which were viscous were stirred further until they were well mixed. One cubic centimeter was then placed into each of two sterile centrifuge tubes; one was used for culturing on Corper's potato medium, Corper and Uyei, (1928), Corper and Uyei, (1929), Corper and Uyei, (1930). The other was used for concentrating by Petroff's sodium hydroxide method, A.S.C.P. The smear resulting was dried, fixed, stained by the Ziehl-Neelson method, (the same as those resulting from the "whiping" and "picking" processes), and examined for acid-fast bacteria.

Results: 1,000 positive sputa.

Method	Number of positives	% of of positives
1. Whipping	902	90.2%
2. Picking	926	92.6%
3. Concentration	980	98.0%
4. Culturing	921	92.1%

Discussion: The concentration procedure of Petroff gave the best results, i.e., 98%. Others, Corper and Uyei, (1928), Corper and Uyei, (1929), and Cunningham and Cummings, (1930), have found the "Culture Medium" to be more efficient. In this study, the worst fault encountered with it, was that the media dried out too often. This work was done in a very dry climate, so that any improper seal or leak of the test tube allowed the medium to dry out. Other disadvantages observed were that quite often the cultures were contaminated, and that it took so long to get the final report. It is, however, a valuable aid, as several sputa were found to be positive with it, when all others were negative. It gave about the same results that the "picking" procedure did.

Although the "whipping" method did not establish quite as many positives as the "picking" method, it did result in finding twenty-four that the "picking" process did not. This shows that it is a valuable aid in examining sputa for acid-fast bacteria. Using it in conjunction with the "picking" method then, should increase one's

efficiency about 2.5%. This being the case, its routine use should quite frequently save the necessity of obtaining another specimen for repeating the examination, or resorting to more expensive and time-consuming methods as concentrating, culturing, or even inoculating guinea pigs. Believing this to be so, it has become a part of the routine procedure for sputum examinations in this laboratory. With inexperienced technicians, the percentage of positives obtained should be higher than that of the "picking" process; as experience does not affect the efficiency of the "whipping" procedure, but it certainly does the "picking" procedure.

Summary and Conclusions

1. The "Whipping Method" for making examinations of sputa for Mybacterium tuberculosis, has been presented.

2. A comparison of its efficiency with other methods, has been proven to be a valuable aid.

3. It is believed that it is especially valuable to an inexperienced laboratory worker, as experience has no affect upon its efficiency.

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REPORT OF THE COMMITTEE ON EDUCATION AND RESEARCH OF THE AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS

June 11, 1940

Since the scope of the Committee on Education and Research is so broad, this report is quite lengthy, but we ask your indulgence, and your careful consideration of the program we wish to inaugurate.

With the attempt to infringe upon our name and insignia by inadequately trained laboratory workers, it behooves us to redouble our efforts to educate physicians and prospective medical technologists to the need for laboratory technicians, trained in the basic sciences, practically directed by pathologists in approved schools, and examined for competency by a recognized Board of Registry, in short to the need and value of A. S. C. P. Registered Medical Technologists.

With this thought before us, we have outlined the following program:

Section I. *Education of Physicians.*

A. Placing before physicians everywhere a description of Medical Technologists and their ideals and aspirations through:
   1. Timely articles in medical journals.
   2. Explanatory exhibits at all local, state, and regional medical meetings.
   3. Individual contact with technologists who strive toward our ideal.

This committee, we believe, can be of real service by formulating model exhibits which can be distributed upon request, and by having available general articles for publication, which can be altered slightly to fit the local needs. This is really not a very great task, and I believe it is a service which would be appreciated since I have received several requests for such material from various parts of the country.

Attached to this report are two model articles for publication in Medical Journals (Forms No. 1 and No. 2) and diagrams of exhibits (Forms No. 3 and No. 4) suitable for display at medical

meetings. If you have not already seen the model exhibits in miniature, presented by this committee, we urge you to examine them later.

Section II. *Education of Medical Technologists.*

A. Members of the A. S. M. T.

We know that many technologists would like to do something for their profession and with only a little stimulation would be anxious to take an active and responsible part in our activities. Model speeches and papers would give them confidence till they could develop their own latent powers.

The attached letter (Form No. 5) could be sent to all state societies announcing the services which the committee would be prepared to offer, and pointing out the opportunities for each individual member to help carry out our national program by presenting these exhibits, papers, etc., or ones of their own composition, in their local communities.

This committee could also furnish suggested programs for local study groups, state conclaves, etc.

Section II. B. *Non-Members of the A. S. M. T.*

1. In connection with the membership committee, a letter might be sent to all registrants, especially new ones, which would point out the benefits of the A. S. M. T. program to the individual: keeping up to date, stimulation of A. S. C. P. speakers, A. M. A. exhibits, fellowship of our own national meetings and inspiration from the same; as well as our collective responsibility to our profession and the dangers of state control and means by which we may combat it.
   (The committee recommends that the Society secure the consent of the Registry to enclose this letter with the Registry's renewal statement, to reduce expenses.)
   Attached to this report is a diagram of an exhibit (Form No. 6) which could be used at state meetings to invite registrants to become A. S. M. T. members.
2. Place exhibits before local and state hospital association meetings.
   Attached to this report is a diagram of an exhibit (Form No. 7) suitable for such a meeting.

Section III. *Education of Non-Registered Technicians.*

These technicians could be reached by local study groups, who could invite them to attend their meetings, and through informative and inspiring programs could interest them in meeting Registry qualifications and applying for registration.

They could also be invited to state meetings, and through speeches and exhibits be made to feel their personal inadequacy for the work they are doing (this should be done by having the program of such high quality that inferior work would be obvious); or if they are qualified to be registered, they should be made to feel the value of such registration to themselves.

Section IV. *Education of Prospective Medical Technologists.*

We should place Registry literature in occupational study groups in high schools and colleges, discussing our aims, qualifications and work with teachers who will be questioned by students and prospective technicians; we could have papers and speeches available to be given to these groups.

Section IV. *Education of the Public,* through

A. Newspaper articles.

Attached to this report is an article (Form No. 9) which could be used as a model.

B. Medical exhibits for the laity, such as "Halls of Health," presented by medical societies in your locality. Attached to this report is a picture (Form No. 10) of such an exhibit which was presented by the Tulsa Roundtable of Medical Technologists at the Hall of Health sponsored by the Tulsa County Medical Society.

This concludes our program for the educational portion of the committee. Since, according to our constitution, this committee is authorized to work in connection with the House of Delegates, we ask for your approval of this program. Because we were not authorized to proceed on a national scale we did try out many of these projects in Oklahoma, and it is a workable program. We believe that it can be carried out at a cost not to exceed $10.00 for the year.

The second portion of our report deals with research. When the Committee on Education and Research surveyed its job for the year, the first question was what are the duties of the committee, and what have the committees done in the past? It was impossible to glean many suggestions from this source, but we did receive one

suggestion, namely, to consider the advisability of presenting plans for an honor society, a Greek letter organization, membership in which would be conferred on members of the American Society who have published a certain number of original papers in the medical journals listed in the "Quarterly Cumulative Index Medicus."

The committee is, and I'm sure we all are, in favor of stimulating personal endeavor to greater heights and in trying to keep out of the ruts that line our daily paths, but we feel that in the days when we are striving to build a strong national organization we cannot be too careful to avoid arousing possible points of contention unless they mean a great deal to the fulfillment of our aims and ambitions.

As you well know, some commercial schools have been fostering state licensing of technicians in an effort to be able to tell their students that they will have a chance to practice in recognized hospitals. We feel, I'm sure, with the Registry, that the qualifications which the A. S. C. P. has set up are not too much to expect of anyone who will share responsibility for human life and that any reduction in these qualifications would be a very definite step backward for laboratory medicine in general and for our profession specifically. So it is imperative that we strive for and maintain a unified front with a definite program which will attract all technicians who are qualified to join our group.

So the question is—Whenever we are considering a new project—will this proposition promote a more unified group of medical technologists, will it take us a step further toward the realization of our avowed objectives, or are their serious possibilities of it arousing factions and splitting our membership into antagonistic groups?

The suggested honor society was proposed, we feel sure, in an effort to stimulate endeavor on the part of medical technologists, and to give new registrants who have just completed their academic and hospital training some new goal toward which to strive.

An honorary Greek letter society should be considered perhaps, although the committee thinks the time is in the far future when such a society would be advisable. Just now we are trying to work with a House of Delegates to enlarge our membership and lower our membership dues. We want to have a firm, well-knit organization. Until we have a much stronger one than we now have, we

do not think an organization within an organization should be started. Of course it is to be expected that an organization has groups in favor of one thing or another but another organization is a very different thing. Therefore we are not in favor of that form of recognition at the present time.

It seems we have no definite way of knowing which members of our organization are doing research. We should have a file or reference to their activities. Many of our most valuable technicians in the field of research do not publish the papers but lay the ground work for their chiefs and co-workers to receive the credit. We feel that this is the class that is to be encouraged most.

The committee wishes to make the following recommendations in regard to the program for fostering research by the members of the American Society of Medical Technologists:

1. That a questionnaire (Form No. 11) attached to this report, or a modification of it, may be sent to the members of the American Society of Medical Technologists, and to new members as they join to aid the Research Committee in selecting members of our Society worthy of special recognition.

The committee also recommends that this questionnaire be sent to the membership along with the statements for dues, to eliminate expense of additional postage.

2. That the Research Committee of next year consider a plan by which outstanding research along the lines of medical technology by members of our Society could be recognized, and suggest that an award or medal is the most practical means of recognition at the present time.

Committee on Education and Research of the American Society of Medical Technologists:

Rowena M. Johnson, Chairman,
Henrietta Lyle,
Christine Seguin,
Faith Dravis,
Margaret Brown.

NEWS AND ANNOUNCEMENTS

MINUTES OF THE AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS EIGHTH ANNUAL CONVENTION

June 10, 11, 12, 1940

MINUTES OF THE BOARD OF DIRECTORS

June 9, 4 p. m.

The Board of Directors was called to order by Bernice Elliott, President, at the Hotel Biltmore, New York City. Those present were Bernice Elliott, Hermine Tate, David Silcock, John Conlin, Henrietta Lyle, Ann Snow, Dorothea Zoll, Annette Callan, and Zana Skidmore.

The major portion of business was the discussion and approval of each paragraph of the Report of the Board of Directors.

A motion was made by Henrietta Lyle that the Society pay the expense of photographs of the exhibits for use in the Journal.

The motion was seconded by Dorothea Zoll and passed.

MINUTES OF THE CONVENTION

June 10, 1940

The convention was called to order by Doris B. Griffiths at 9:30 a. m.

After the invocation by the Reverend Thomas A. Sparks, announcements were made including the following appointments to the Resolution Committee

Mary Beatrice Leisman, Chairman,

Lucille Wallace,

Sr. M. Celeste Waynant.

The President's Address was delivered by Bernice Elliott and the scientific program began.

At the end of the morning session Bernice Elliott called the convention to order to arrange the selection of delegates to be accepted by the Credentials Committee, Marian A. Baker, Chairman.

MINUTES OF THE HOUSE OF DELEGATES

June 11, 1940

The House of Delegates was called to order by Bernice Elliott.

Bernice Elliott called for the minutes of the 1939 convention.

John Fitzgerald moved we omit reading the minutes.

There were many seconds and the motion passed.

Henrietta Lyle moved that the minutes of the 1939 convention be approved as published. This motion was seconded and passed.

Amendments to the Constitution were then considered as follows:

By-Laws, Article II, Section 1. Strike out "state." Motion was made to approve this amendment, seconded and passed.

By-Laws, Article III, Section 1. Change to read: "Each affiliated society in good standing shall be entitled to one delegate in the House of Delegates for every twenty-five (25) members or fraction thereof, of such society who are at the time of the meeting of the House of Delegates active members in good standing in the American Society of Medical Technologists, except in the affiliated society or societies shall be entitled to at least one and not more than six delegates for any one state. The affiliated society or societies shall provide the method of electing or appointing its delegates." A motion to approve this amendment was seconded and passed.

Lucille Wallace made a motion that we receive the report of the Educational and Research Committee before considering other amendments or as part of the discussion. The motion was seconded by Arthur Brice. Motion passed.

The Report of the Educational and Research Committee was read by Rowena Johnson, Chairman. Arthur Brice moved the adoption of the Report of the Educational and Research Committee. Motion was seconded and passed.

By-Laws, Article VIII, Section 3. Cross out the last sentence and insert the following sentences: "The Educational and Research Committee shall consist of six (6) members. The members shall serve for three (3) years, and two (2) members shall be appointed annually, except that in 1940, six (6) members shall be appointed, two (2) to serve for one (1) year, two to serve for two (2) years and two (2) to serve for three years. This committee shall devise and direct the education and research activities of this society under

the general direction of the House of Delegates and the Board of Directors." Carolyn Weitzel moved the adoption of this amendment. Motion was seconded and lost.

By-Laws, Article VIII, Section 3. Alternate proposal of sentence to insert: "The Education Committee and the Research Committee shall each consist of six (6) members. The members shall serve for three (3) years and two (2) members shall be appointed annually except that in 1940 six (6) members shall be appointed, two (2) to serve for one (1) year, two (2) to serve two (2) years, and two (2) to serve three (3) years. Each committee shall work under the general direction of the House of Delegates and the Board of Directors, the Education Committee to devise and direct the educational activities, and the Research Committee to devise and direct the research activities of the society." Motion was made to adopt this amendment. Seconded and passed.

By-Laws, Article I, Section 3. Strike out "Four dollars ($4.00)" and insert "Three dollars and twenty-five cents ($3.25)".

Evelyn Jardine moved that our annual dues remain four dollars ($4.00)". Motion seconded and passed.

By-Laws, Article VII, Section 5 (additional): "A sum of two hundred dollars ($200.00) shall be provided annually to be divided proportionally, according to their distance from the annual meeting place, between the following officers: President, President-Elect, Recording Secretary, and Treasurer. The share of any of these officers not attending the annual meeting shall be returned to the Treasury of the Society." A motion to adopt this amendment was made, seconded, and passed.

Mary Leisman made a motion that we postpone committee reports and proceed with the election. The motion was seconded and passed.

Carolyn Weitzell moved that we elect the Nominating Committee at the same time as the general election. Motion was seconded.

A motion was made to amend the motion to elect the Nomination from the floor. The motion to amend was seconded and passed. The amended motion passed.

Frieda Claussen made a motion that two members be elected to the Advisory Board of the Board of Registry to be chosen from the floor; the first year, one (1) to serve one (1) year and one (1)

to serve two (2) years and one (1) elected for two (2) years at each annual election thereafter. Motion seconded and passed.

Frieda Claussen moved that the President serve this year as Board member and that the President-Elect serve two (2) years as Board member, and that the President-Elect to be the Board member thereafter. The motion was seconded and passed.

Bernice Elliott appointed tellers: Dorothea Zoll, Henrietta Lyle, Evelyn Jardine.

The report of the Nominating Committee was read. Emily Smith moved the approval of the report. The motion was seconded and passed.

Carolyn Weitzell made a motion that discussion be limited to five (5) minutes on any motion. The motion was seconded and passed.

Marian Baker made a motion to limit discussion of any individual to one (1) minute. The motion was seconded and passed.

Bernice Elliott appointed Arthur Brice Timekeeper.

Bernice Elliott announced that nominations for members of the Nominating Committee were in order.

Nominations: Rose Matthaei, Rowena Johnson, Ann Snow.

John Fitzgerald made a motion that this ticket of three (3) be elected. The motion was seconded and passed.

Ballots were passed by the tellers. The ballots were collected and the tellers retired.

Evelyn Jardine made a motion that we discard our present Laboratory uniform by the Marvin-Neitzel Company. The motion was seconded and passed.

Report of the tellers: President-Elect: Lawrence Ray; Recording Secretary: Marian Baker; Treasurer: Hermine Tate; Board of Directors: Frieda Claussen, Bernice Elliott; Advisory Board: Sr. M. Jeannette Bodoh.

The remainder of the session was given over to committee reports. All reports were approved by the House of Delegates.

Hermine Tate moved we adjourn. The motion was seconded and passed.

MINUTES OF THE HOUSE OF DELEGATES
June 12, 1940

Bernice Elliott called a meeting of the House of Delegates; twenty-six were present.

John Fitzgerald made a motion that we approve five hundred dollars ($500.00) for the publication of the Journal of the American Society of Medical Technologists. The motion was seconded and passed.

Marian Baker made a motion that the American Society of Medical Technologists be placed on record as disapproving the activities of groups of laboratory workers who have not met with the standards and qualifications of this body. The motion was seconded and passed.

Cecelia Kortuem made a motion to adjourn. The motion was seconded and passed.

MINUTES OF THE BOARD OF DIRECTORS
June 12, 1940

Bernice Elliott called the meeting to order.

Henrietta Lyle made a motion that we meet the first three (3) days of the American Medical Association 1941 meeting.

Bills were presented and approved.

A motion was made to adjourn. The motion was seconded and passed.

AWARDS, ANNUAL SESSION, NEW YORK CITY, JUNE, 1940:

First Prize—Gold medal for paper, Cecelia Kortuem, St. Vincent's Hospital, Chicago, Ill.

Second Prize—Silver medal for exhibit, Phyllis Stanley, Presbyterian Hospital, Newark, N. J.

Third Prize—Bronze medal for paper, Evelyn M. Jardine, Mary Hitchcock Hospital, Hanover, N. H.

Honorable Mentions—For paper, Marian Baker, Lufkin, Texas; for paper, Hermine Tate, Charity Hospital, Lafayette, La.

Guest Exhibitors, Special Mention—Army Medical Museum, Washington, D. C., Lt. Col. J. E. Ash, F. M. Kramer and Associates (Pathologic Exhibit). Jewish Memorial Hospital, New York City, Drs. Weisman, Angrist and Schwartz (Spermatozoa).

AWARDS COMMITTEE,
Dr. Davidsohn,
Dr. Larimore,
Annette Callan.

COMMITTEE APPOINTMENTS FOR FISCAL YEAR, 1940-41:

Program Committee—

Dorothea Zoll, Philadelphia, Pa., Chairman.
Frieda Claussen, St. Paul, Minn.
Evelyn Jardine, Hanover, N. M.
Rachel Lehman, Evansville, Ind.

Scientific Exhibits Committee—

Marian Baker, Lufkin, Texas, Chairman.

Entertainment Committee—

Mary B. Leisman, Louisville, Ky., Chairman.

Publicity Committee—

Marian Gianniny, Philadelphia, Pa., Chairman.

Sisters Reservations and Entertainment Committee—

Sister M. Eulalia Rothermel, Cleveland, Ohio.

Education Committee—

Rowena Johnson, Tulsa, Okla., Chairman.

Nominating Committee—

Rose Matthaei.
Rowena Johnson.
Ann Snow.

Kentucky

Annual report of the Kentucky Society of Medical Technologists.

During the year since the last national meeting, the Kentucky Society of Medical Technologists has become an affiliated society of the American Society of Medical Technologists. Our charter is No. II. The Louisville and Lexington societies are affiliated with the state society. Our membership has increased to fifty-three, and we are conducting an intensive campaign for members for both the state and national societies.

Our second annual meeting was held in Bowling Green, Kentucky, on September 14, 1939. We had a very interesting and instructive program. Among the speakers were Dr. Irvin Abell, Jr., Dr. Harry S. Andrews, and Dr. Morris Thompson, all of Louisville. A symposium on laboratory procedures and problems followed the papers. During the business session it was decided that the society

should become incorporated under the laws of Kentucky in order to protect its name and interests. The matter is at present in the hands of our lawyer. The following officers were elected:

President, Miss Mary B. Leisman, M.T., Louisville, Ky.

Vice-President, Robert D. Greene, Lexington, Ky.

Secretary, Miss Mary Elizabeth Conkling, Louisville, Ky.

Treasurer, Mr. David S. G. Silcock, Versailles, Ky.

Mr. O. M. Alton of Louisville, and Mr. Nelson Blankenship of Bowling Green were elected to the vacancies on the Executive Board.

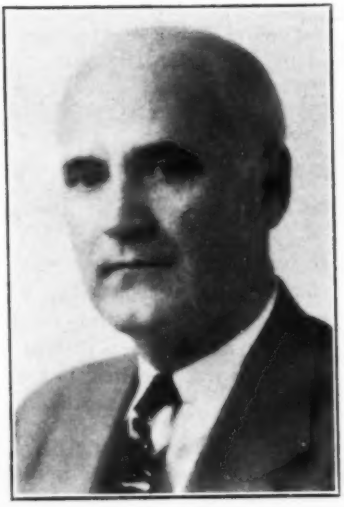
A campaign to acquaint the doctors with our society, its aims and aspirations, has been a vital part of our year's activity. We have had an exhibit at every major medical meeting in the state. We plan to have an especially attractive one at the Southern Medical Meeting here in Louisville next November, and intend to invite the other societies throughout the South to collaborate with us in this project.

EXAMINATION FOR APPOINTMENT AS COMMISSIONED OFFICERS IN THE MEDICAL CORPS OF THE U. S. NAVY

The next examination for doctors of medicine desiring to enter the Medical Corps of the United States Navy will be held on August 19, 1940 at the following Naval Medical Department activities:

U. S. Naval Hospital, Chelsea, Massachusetts.
U. S. Naval Hospital, Brooklyn, New York.
Norfolk Naval Hospital, Portsmouth, Virginia.
U. S. Naval Hospital, Pensacola, Florida.
U. S. Naval Hospital, San Diego, California.
Naval Medical Center, Washington, D. C.
U. S. Naval Hospital, Newport, Rhode Island.
U. S. Naval Hospital, Philadelphia, Pennsylvania.
U. S. Naval Hospital, Charleston, South Carolinia.
U. S. Naval Hospital, Great Lakes, Illinois.
U. S. Naval Hospital, Mare Island, California.
U. S. Naval Hospital, Puget Sound, Bremerton, Washington.

HENRIETTA LYLE. M.T.
President A. S. M. T., 1940-41



LAWRENCE O. RAY. President-Elect

Graduates of Class "A" medical schools who have had an internship in a civilian hospital and who are physically and professionally qualified may be commissioned in the permanent Medical Corps of the Navy as Assistant Surgeons with the rank of Lieutenant (junior grade). Applicants must be less than thirty-two (32) years of age at the time they receive their commissions, citizens of the United States, physically qualified for appointment as officers in the Medical Corps and must demonstrate their professional qualifications by competitive written, oral and practical examinations. The professional examination will embrace the subjects of: (1) General Medicine, (2) General Surgery, (3) Obstetrics and Gynecology, and (4) Preventive Medicine and Medical Jurisprudence.

The pay and allowances for Assistant Surgeons with the rank of Lieutenant (junior grade) in the Medical Corps of the Navy is $2,699 per year if the officer has no dependents, and $3,158 per year if he has dependents.

Additional information regarding physical requirements, etc., may be obtained by addressing a letter to the Bureau of Medicine and Surgery, Navy Department, Washington, D. C. Applications must be completed and received in the Bureau of Medicine and Surgery prior to August 1, 1940 in order that authorization may reach the applicant in sufficient time for him to appear for examination on August 19, 1940.

ANNOUNCEMENT OF A STUDY TO EVALUATE ORIGINAL SEROLOGIC TESTS FOR SYPHILIS

More than five years ago the Committee on Evaluation of Serodiagnostic Tests for Syphilis, in cooperation with the United States Public Health Service, conducted a study to evaluate original serologic tests for syphilis or modifications thereof in the United States. The results of this study were published shortly after the investigation was completed.[1]

Consideration is now being given by the Committee to the organization of a second evaluation study of original serologic tests

for syphilis or modifications thereof within the next year. If the need for an investigation of this kind seems to justify the cost, invitations will be extended to the authors of such serologic tests who reside in the United States, or who may be able to participate by the designation of a serologist who will represent them in this country. The second evaluation study will be conducted utilizing methods comparable to those employed in the first study.[2]

Serologists who have an original serologic test for syphilis or an original modification thereof and who desire to participate in the second evaluation study should submit their applications not later than October 1, 1940. The applications must be accompanied by a complete description of the technic of the author's serologic test or modification. All correspondence should be directed to the Surgeon General, United States Public Health Service, Washington, D. C.

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[1]Ven. Dis. Inform., Washington. June, 1935, 16: 189.
J.A.M.A., Chicago. June 8, 1935, 104: 2083.

[2]J.A.M.A., Chicago. Dec. 1, 1934, 103: 1705.

EXHIBITS—ANNUAL SESSION, NEW YORK CITY, JUNE, 1940